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RESEARCH ARTICLE

IN SILICO MODELLING OF ENGINEERED BUFORIN II

[1, 2]Golla Kamala, [2]Vutukuru, S. S. and [1,*]Chand Pasha

[1]Department of Microbiology, Nizam College, Osmania University, Hyderabad, Telangana, India
[2]Department of Biotechnology, Sreenidhi Institute of Science and Technology, Hyderabad, India

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ABSTRACT

Protein engineering-based drug designing is quite interesting research area for invention of peptide based drugs. The current study is aimed to engineer BuforinII antimicrobial peptide by Mechanism based protein engineering. Template BuforinII was obtained from NCBI database and its similar sequences were retrieved by using Blast P. Protein engineering resulted in a total of 200 variable peptides. 3D model developed for 200 variable peptides and BuforinII by using PEPFOLD server. The developed models were evaluated by RAMPAGE and Q-Mean servers. Based on the Ramachandran Plot, Z-score value and SVM-score of DNA binding studies, the P11A/V12P variant was found to be highly stable and more DNA binding compared with wild and remaining variants. The developed variants can be used for antimicrobial studies in laboratory.

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INTRODUCTION

Antimicrobial peptides have received attention as alternative therapeutic agents to overcome the limitations of current drugs (Wojciech, 2005) like antibiotic resistance. So it is the time for discover novel drugs with high potency and lower risk of resistant strains development (Asoodeh *et al.,* 2012). Antimicrobial peptides (AMPs) are produced as a fist line of defence by unicellular to multicellular organisms (Fernanda *et al.,* 2013). AMPs have broad spectrum antimicrobial activity including on antibiotic resistance bacteria (Wataru and Mitsuyoshi, 2013; Jun *et al.,* 2013). Antimicrobial peptides majorly disrupt bacteria membrane, few bind DNA/RNA and few interfere intracellular process. Buforin I is a 39 amino acid antimicrobial peptide, isolated from the stomach surface tissue of Asian toad *Bufo bufo garagrizans*. Buforin II derived from buforin I, is more potent antimicrobial peptide containing 21 amino acids (Park *et al.,* 2000). Buforin I and II are histone protein H2A like molecules (Elmore, 2012). BuforinII efficiently cross the lipid bilayer without damage of the membrane (Kobayashi *et al.,* 2000). BuforinII can interact cooperative manner with bacterial membrane and involved in the formation of rapid dissociate transient toroidal pores (Elmore, 2012).

Corresponding author: Chand Pasha
Department of Microbiology, Nizam College, Osmania University, Hyderabad, Telangana, India.

Buforin II penetrate cell membrane and strongly bind to DNA and RNA, destroying RNase/DNase activity. Buforin II, amphipathic structure consist of helix-hinge-helix regions. Initially N-terminal 4 amino acids were participating in random coils, 5-10 a.as involved in primary helix, 12-21 a.as forms second helix, finally this two helix were connected with proline 11 hinge region (Park *et al.,* 2000). The present study is aimed to design BuforinII peptide variants by protein engineering for improved membrane permeability, DNA binding for efficient antimicrobial activity.

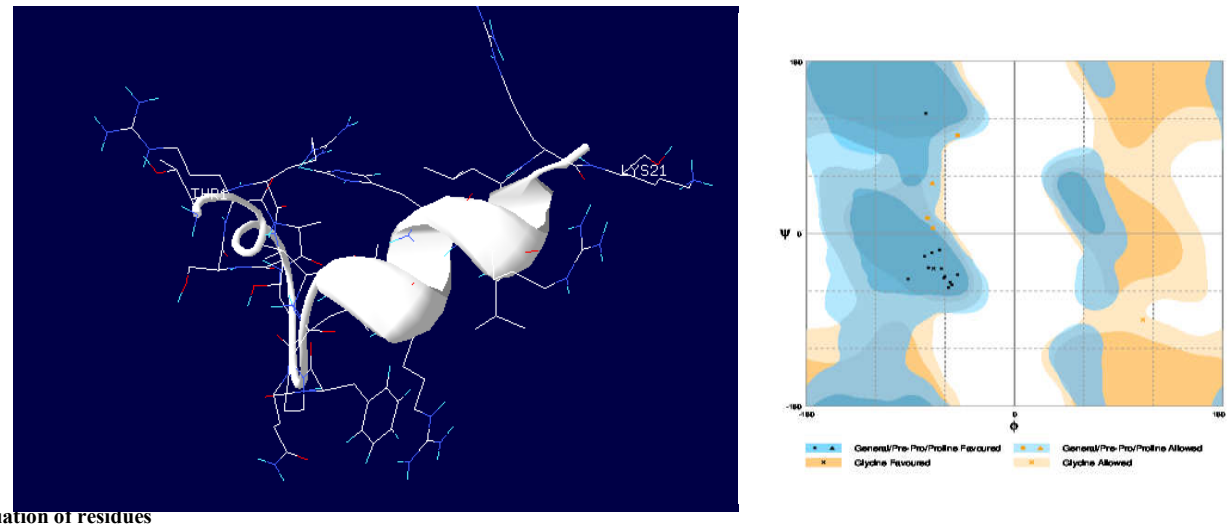
MATERIALS AND METHODS

Retrieval of sequence

Sequence of BuforinII an antimicrobial agent was obtained from NCBI database (https://www.ncbi.nlm.nih.gov/protein/AAB36002.1). FASTA sequence was used for search in non-redundant (nr) data base using NCBI BLASTp algorithm.

Multiple sequence alignment

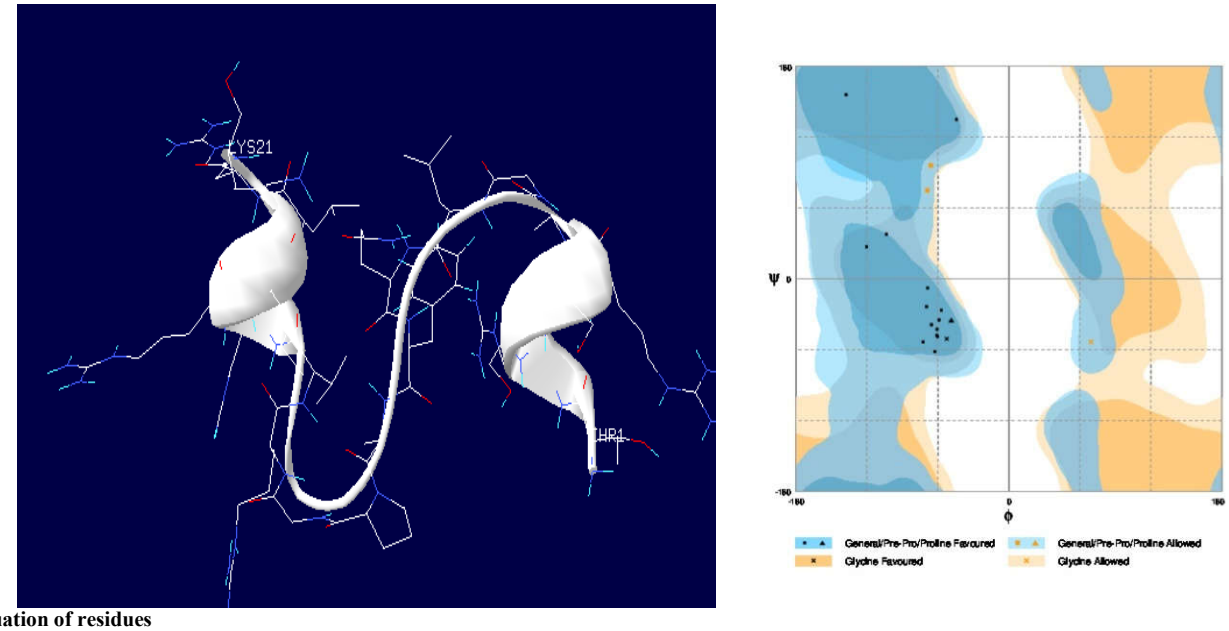
BuforinII and its similar sequences were used in Multiple Sequence Alignment (MSA) through Clustal Omega online tool (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Fabian *et al.,* 2011) Template and target FASTA sequences were submitted in the work space area with default parameters.



Evaluation of residues

Residue [8: LEU] (-49.09, 102.49) in Allowed region;Residue [11: PRO] (-70.89, 52.73) in Allowed region; Residue [12: VAL] (-75.15, 16.23) in Allowed region; Residue [13: GLY] (111.16, -89.96) in Allowed region; Residue [14: ARG] (-70.40, 5.58) in Allowed region; Number of residues in favoured region (~98.0% expected): 14 (73.7%); Number of residues in allowed region (~2.0% expected): 5 (26.3%); Number of residues in outlier region: 0 (0.0%)

Fig. 1. Wild Buforin II (a) PDB structure (b) Ramachandran plot



Evaluation of residues

Residue [7: GLY] (69.55, -53.47) in Allowed region; Residue [8: LEU] (-65.90, 95.90) in Allowed region; Residue [9: GLN] (-68.80, 74.82) in Allowed region; Number of residues in favoured region (~98.0% expected): 16 (84.2%); Number of residues in allowed region (~2.0% expected): 3 (15.8%); Number of residues in outlier region: 0 (0.0%)

Fig. 2. Buforin II variant P11A and V12P (a) PDB structure (b) Ramachandran plot

Aligned sequences were inspected, gaps and insertions were manually removed. It is programmed to align the sequences from high to least similarity.

Mechanism-based protein engineering

As there is no much variation in multiple sequence alignment, mechanism based protein engineering was carried out. Glycine 7, Proline11, Valine 12, Arginine 20 and truncation of N-terminal amino acids were used for engineering as their combination play important role transmembrane, permeability and antimicrobial activity (Yang *et al.,* 2011). In our study the above modifications were used to generate 200 engineered variant peptides.

Modeling of BuforinII variants

Homology modelling of engineered peptides was done using PEP-FOLD (Kumar and Singh, 2016; Brzozowska *et al.,* 2015) work space. Once automated template was identified, modelling carried out using same server and 1-5 optimized models were generated.

Validation of model: The models were refined with energy minimization of Swiss PDB viewer (Kuldeep and Satpal 2013). The models were further validated using Q-mean server (Benkert *et al.,* 2008), and RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) Nazanin *et al.,* 2016; Ingale, 2010).

Table 1. BuforinII and its variants with Z-score values and hydrophobicity

S.No	Buf and its 20 stable variants-sequence A.A																						Z-Score	SVM-score
1	Wild	T	R	S	S	R	A	G	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	21	-0.77	1.013
2	P11A	T	R	S	S	R	A	G	L	Q	F	A	V	G	R	V	H	R	L	L	R	K	21	-1.51	2.421
3	G7P	T	R	S	S	R	A	P	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	21	0.93	0.492
4	G7P/ P11A	T	R	S	S	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	R	K	21	-1.92	1.985
5	T1-G7P/P11A	-	R	S	S	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	R	K	21	-1.6	0.789
6	T1-/G7P	-	R	S	S	R	A	P	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	20	0.85	-0.441
7	T1, R2-/G7P	-	-	S	S	R	A	P	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	19	-1.4	-0.845
8	T1, R2,S3-/G7P	-	-	-	S	R	A	P	L	Q	F	P	V	G	R	V	H	R	L	L	R	K	18	1.44	-0.506
9	T1, R2,S3,S4-/G7P	-	-	-	-	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	R	K	17	0.62	0.984
10	T1-/G7P/ V12P	-	R	S	S	R	A	P	L	Q	F	P	P	G	R	V	H	R	L	L	R	K	20	0.4	-0.108
11	P11A/V12G	T	R	S	S	R	A	G	L	Q	F	A	G	G	R	V	H	R	L	L	R	K	21	-0.49	2.476
12	P11A/V12P	T	R	S	S	R	A	G	L	Q	F	A	P	G	R	V	H	K	L	L	R	K	21	-0.17	2.608
13	G7P/P11A	T	R	S	S	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	R	K	21	-0.36	1.985
14	G7P/P11A/V12P	T	R	S	S	R	A	P	L	Q	F	A	P	G	R	V	H	R	L	L	R	K	21	-1.7	1.887
15	R20K	T	R	S	S	R	A	G	L	Q	F	P	V	G	R	V	H	R	L	L	K	K	21	0.45	1.126
16	G7P/ R20K	T	R	S	S	R	A	P	L	Q	F	p	V	G	R	V	H	R	L	L	K	K	21	0.79	0.661
17	G7P/P11A/ R20K	T	R	S	S	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	K	K	21	-1.25	2.225
18	T1, R2,S3,S4-R20K	-	-	-	-	R	A	G	L	Q	F	P	V	G	R	V	H	R	L	L	K	K	17	2.09	-0.005
19	T1, R2-G7P/ R20K	-	-	S	S	R	A	P	L	Q	F	P	V	G	R	V	H	R	L	L	K	K	19	-0.22	-0.669
20	T1-G7P/P11A/ R20K	-	R	S	S	R	A	P	L	Q	F	A	V	G	R	V	H	R	L	L	K	K	20	-2.72	1.113

DNA Binding studies: The stable engineered BufII variants DNA binding efficiency calculated through SVM-score using DNA Binder (http://www.imtech.res.in/raghava/ dnabinder/index.html) (Manish *et al.,* 2016).

RESULTS

Sequence collection

Buforin II antimicrobial peptide present in Bufo bufo, Asian toads, gargarizans, stomach and consist of 21 amino acids (TRSSRAG LQFPVGRVHRLLRK). NCBI BLASTp of BuforinII (aab36002.1) resulted 50 similar sequences. The following are the retrieved sequenced and their IDs.

>AAB36002.1, >P55897.1, >XP_006931497, >XP_006642425, >P59890.2|H2A_HI, >XP_010830603, >CAG11544.1, >XP_004331957, >AEJ86583.1, >AEJ86582.1, >XP_006220149, >CAG13258.1, >XP_007445527, >XP_007074560, >XP_007074561, >XP_005815224, >XP_010331020, >XP_009176317, >XP_004316340, >AAI51735.1, >4KHA, >XP_004599893, >CAG02874.1, >XP_003979888, >CAF97260.1, >CAF95804.1, >AIE40056.1, >AIE40057.1, >AIE40058.1, >AIE40059.1, >XP_006040926, >EFB20865.1, >1S32, >EFB20877.1, >XP_010621337, >ELR58890.1, >XP_010331019, >XP_003980056, >1AOI, >EFB21589.1, >XP_008403546, >XP_006058485, >XP_006764351, >XP_006220031, |3KWQ, XP_010357557, XP_008539721, XP_009048394, XP_009067138, XP_009047723.

Multiple sequence alignment

Multiple sequence alignment of 50 peptides using Clutsal omega revealed highly conservation in sequence studies. Hence emphirism based protein engineering cannot be used and peptides were modified with mechanism based protein engineering approach.

Mechanism based protein engineering

N-terminal truncation, 7, 11, 12 and 20th amino acids modified to give 200 BuforinII variants (data not shown).

3D structures development and validation

For 200 engineered BuforinII variants PDB structures were developed using PEP FOLD server. Q-Mean server was used for determining model reliability and RAMPAGE for calculating Ramachandran plot. BuforinII V- P11A/ V12P model had highest number of residues in the most favoured regions compared to the wild BuforinII (Fig: 1 and 2). Out of 200 BuforinII variants, 20 highly reliable 3D structures variants were used for Ramachandran plots and Z-score values analysis. These 20 sequences details are given in table1. BuforinII P11A/ V12P variant was the relatively lowest (-0.17) Z- score value and increased SVM-score (2.608) indicating high stability and more efficient DNA binding compared with the wild BuforinII (-0.77) and (1.013).

DISCUSSION

BuforinII, 21 amino acids containing potent antimicrobial peptide is studied for protein engineering (Yang *et al.,* 2011). BuforinII efficiently cross lipid bilayer without membrane permeabilization8 and binds strongly to DNA/ RNA (Kobayashi *et al.,* 2004; Takeshima *et al.,* 2003). Proline is known for binding of two α-helixes and also involving penetration of cell membrane but Proline modified with Alanine and valine replaced with Proline (P11A)/ (V12P) was found highest membrane permeability and DNA binding(Yang *et al.,* 2011). Insilico DNA binding efficiency expressed in SVM scores, increased values result tight DNA binding (Pompeani *et al.,* 2008). In our present study, modifications were done in BuforinII at 7, 11, 12, 20 and N-terminal, the engineered peptides were modelled for stability and DNA binding studies. Out of the variants developed, P11A/ V12P was found to be highly stable and more efficiently interact with DNA. It is permeabilizing through membrane and binding to DNA compared with the wild as experimentally proved in Yang X *et al.,* 2011 studies. The present protein engineering and *In silico* structure development, and validation conforms drug development

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